REMARKS

Reconsideration of the above-identified patent application in view of the amendment above and the remarks below is respectfully requested.

Claims 29-30 have been canceled in this paper. Claims 1-28 have been amended in this paper. No new claims have been added in this paper. Therefore, claims 1-28 and 31 are pending. Of these claims, claim 31 is withdrawn as being directed at a non-elected invention. Accordingly, claims 1-28 are under active consideration.

Claims 1-30 stand rejected under 35 U.S.C. 112, second paragraph, "as being indefinite for failing to particularly point and distinctly claim the subject matter which applicant regards as the invention." In support of the rejection, the Patent Office states the following:

> Claims 1-29 are indefinite because the claims do not recite the basic steps of the claimed method in a positive, active fashion. The claims are drawn to "detection of cytosine methylation...characterized in that...the DNA to be investigated...is reacted...is amplified...is separated.." which are not active process steps. See Ex parte Erlich, 3 USPQ2d, 1011 (BPAI 1986): "Method claims need not recite all operating details but should at least recite positive, active steps so that claim will set out and circumscribe particular area with reasonable degree of precision and particularly and make clear what subject matter claims encompass, as well as make clear subject matter from which others would be precluded."
>
> Claims 1-29 recite the limitation "the pretreated DNA" in step b line 1 of Claim 1. There is insufficient antecedent basis for this limitation in the claim. There is no pretreated DNA in step a. It is suggested that the claim be amended to incorporate pretreated DNA in step a in order to have antecedent basis.
>
> Claims 1-29 recite the limitation "the primer extension product" in step c line 1 of Claim 1. There is insufficient antecedent basis for this limitation in the claim. There is no primer extension product in step a or b. It is suggested that the claim be amended to

incorporate primer extension product in step a or b in order to have antecedent basis.

Claims 1-29 are indefinite over the phrase "is hereby characterized in that" in lines 1-2 of Claim1. This phrase makes the metes and bounds of the claims unclear because it is not clear if the method is "consisting of", "consisting essentially of," or "comprising" steps a-e of Claim 1. It is noted that the claims are being interpreted broadly as "comprising" for the art rejections presented below.

Claims 1-29 are unclear over the phrase "at least one primer, whose 5'-end is joined with a probe via a linker (Scorpion primer)" in claim 1 line 7. The claims contain information in parentheses, i.e. (Scorpion primer). Parentheticals make the claim indefinite because it is unclear whether the information in the parentheses has the same, less, or more weight as the rest of the claim language.

Claim 2 is indefinite over the phrase "produced with a bisulfite" in line 2. It is unclear from the claim if the bisulfite is the chemical or enzyme that is reacted with the DNA from step a of Claim 1 or if the "produced with a bisulfite" is a further step of step a.

Claim 3 is indefinite over the phrase "produced by means of a cytidine deaminase" in line 2. It is unclear from the claim if the cytidine deaminase is the chemical or enzyme that is reacted with the DNA from step a of Claim 1 or if the "produced by means of a cytidine deaminase" is a further step of step a.

Claim 3 is indefinite over the phrase "the unmethylated cytidine reacts more rapidly than methylated cytidine". This phrase is unclear because there are no cytidine (methylated or unmethylated) in claim 1. Further it is unclear if this limitation is a limitation of the claims because there is no active step.

Claim 6 is indefinite over the phrase "probe bears" because it is unclear what the metes and bounds of the phrase "probe bears" encompasses. It is not clear how the probe "bears" two signal components, if this phrase means the probe comprising two signal components or some other undefined relationship.

Claim 7 is unclear over the phrase "involve a quencher-fluorescent dye pair". It is not clear [what are] the metes and bounds

of the phrase. It is not clear if the phrase means that the two signal [components comprise] are a quencher-fluorescent dye pair or if these two signal components have some undefined relationship to the quencher-fluorescent dye pair.

A broad range or limitation together with a narrow range or limitation that falls within the broad range or limitation (in the same claim) is considered indefinite, since the resulting claim does not clearly set forth the metes and bounds of the patent protection desired. See MPEP §2173.05(c). Note the explanation given by the Board of Patent Appeals and Interferences in *Ex parte Wu*, 10 USPQ2d 2031, 2033 (Bd. Pat. App. & Inter. 1989), as to where broad language is followed by "such as" and then narrow language. The Board stated that this can render a claim indefinite by raising a question or doubt as to whether the feature introduced by such language is (a) merely exemplary of the remainder of the claim, and therefore not required, or (b) a required feature of the claims. Note also, for example, the decisions of *Ex parte Steigewald*, 131 USPQ 74 (Bd. App. 1961); *Ex parte Hall*, 83 USPQ 38 (Bd. App. 1948); and *Ex parte Hasche*, 86 USPQ 481 (Bd. App. 1949). In the present instance, claim 8 recites the broad recitation the secondary structure of the probe, and the claim also recites particularly by a hairpin shape which is the narrower statement of the range/limitation.

Claims 11-12 recites the limitation "the probe and another oligonucleotide" in line 2 of claim 11. There is insufficient antecedent basis for this limitation in the claim. It is suggested that the claim be amended to correct antecedent basis.

Claims 11-12 are indefinite over the phrase "each bears" because it is unclear what the metes and bounds of the phrase "each bears" encompasses. It is not clear how the probe "bears" two signal components, if this phrase means the probe comprising two signal components or some other undefined relationship.

Claims 11-13 recites the limitation "the signal components" in line 3 of claim 11. There is insufficient antecedent basis for this limitation in the claim. It is suggested that the claim be amended to e.g. "the at least one signal component" to correct antecedent basis.

Claim 12 recites the limitation "the two signal components" in line 1-2. There is insufficient antecedent basis for this limitation in

the claim. It is suggested that the claim be amended to e.g. "the at least one signal component" to correct antecedent basis.

Claim 13 recites the limitation "the spatial separator" in line 2. There is insufficient antecedent basis for this limitation in the claim. It is suggested that the claim be amended to e.g. "the probe and the another oligonucleotide are spatially separated" to correct antecedent basis.

Claim 13 is unclear over the phrase "assured by a duplex structure". It is unclear [what are] the metes and bounds of the phrase because it is not clear how inactive the probe and the other oligonucleotide must be to be "assured".

Claims 14-16 recite the limitation "the probe and another oligonucleotide" in line 2 of claim 14. There is insufficient antecedent basis for this limitation in the claim. It is suggested that the claim be amended to correct antecedent basis.

Claims 14-16 recite the limitation "the signal components" in line 3 of claim 14. There is insufficient antecedent basis for this limitation in the claim. It is suggested that the claim be amended to e.g. "the at least one signal component" to correct antecedent basis.

Claim 16 recites the limitation "the other oligonucleotide" in line 2. There is insufficient antecedent basis for this limitation in the claim. It is not clear if "the other oligonucleotide" is the same as the "another oligonucleotide" in Claim 14 or if "the other oligonucleotide" is an additional oligonucleotide. It is suggested that the claim be amended to e.g. "the another oligonucleotide" to correct antecedent basis.

Claim 17 is unclear because it is unclear where the several sequences are simultaneously amplified should be placed in the method of Claim 1. It is not clear if these sequences are amplified after detection because there is no step in Claim 1 which "amplifies".

Claims 18-20 are unclear because it seems to be limiting an amplification step of Claim 1, however, there are no amplification steps in Claim 1.

Claims 19-21 are indefinite over the phrase "primer bear" because it is unclear what the metes and bounds of the phrase "primer

bear" encompasses. It is not clear how the primer "bear" two signal components or probes, if this phrase means the primer comprising two signal components the primers comprise a probe or some other undefined relationship.

Claims 22-28 are unclear over the phrase in the parentheses. The claims contain information in parentheses, i.e. ("methyl hairpin"), ("MSP methyl hairpin"), ("heavy methyl hairpin"), ("methyl duplex"), ("MSP methyl duplex"), ("heavy methyl duplex"), or ("quantitative methyl hairpin")...Parentheticals make the claim indefinite because it is unclear whether the information in the parentheses has the same, less, or more weight as the rest of the claim language.

Claims 29-30 provides for the use of the method of claim 1 or the use of scorpion primers for methylation analysis, but, since the claim does not set forth any steps involved in the method/process, it is unclear what method/process applicant is intending to encompass. A claim is indefinite where it merely recites a use without any active, positive steps delimiting how this use is actually practiced.

Claims 29-30 are rejected under 35 U.S.C. 101 because the claimed recitation of a use, without setting forth any steps involved in the process, results in an improper definition of a process, i.e., results in a claim which is not a proper process claim under 35 U.S.C. 101. See for example *Ex parte Dunki*, 153 USPQ 678 (Bd. App. 1967) and *Clinical Products, Ltd. v. Brenner*, 255 F. Supp. 131, 149 USPQ 475 (D.D.C. 1966).

Claims 22-24 are indefinite because it is unclear how each differs to further limit the parent. Each of these claims comprises the same steps however the preamble describes a different amplification process is taking place.

Claims 25-27 are indefinite because it is unclear how each differs to further limit the parent. Each of these claims comprises the same steps however the preamble describes a different amplification process is taking place.

Insofar as the subject rejection relates to claims 29 and 30, Applicants respectfully submit that the rejection is moot in view of Applicants' cancellation herein of claims 29 and 30. Insofar as the subject rejection relates to claims 1-28, Applicants respectfully traverse the subject rejection.

Claims 1-28 have been amended extensively in this paper. Applicants respectfully submit that one of ordinary skill in the art, after having read the present specification, would understand the metes and bounds of the claims.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claims 1-2 and 4-30 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Eads et al. (Nucleic acids Research 2000 Vol. 28 p. 32) in view of Solinas et al. (Nucleic acids Research 2001 Vol. 29 p. e96)." In support of the rejection, the Patent Office states the following:

> Eads et al. teaches a method for detecting of cytosine methylation (abstract).
>
> With regard to Claim 1 step a, Eads et al. teaches reacting the DNA with a chemical (e.g., sodium bisulfite) to change unmethylated cytosine to uracil (p. ii 1st column last full sentence). With regard to Claim 1 step b, Eads et al. teaches a PCR amplification with a polymerase, at least one primer, and a probe (p ii 2nd column Methylight primer and probe sequences and Figure 1). However, Eads et al. does not teach that the primer is joined with a probe via a linker. With regard to Claim 1 step c-e, Eads et al. teaches separating the primer strand and detection whether or not hybridization of the probe has occurred (figure 1).
>
> With regard to Claim 2, Eads et al. teaches reacting the DNA with sodium bisulfite (p. ii 1st column last full sentence).
>
> With regard to Claims 4-5, Eads et al. teaches a method of MSP RT-PCR (p. ii 1st column last paragraph and 2nd column 1st paragraph).

With regard to Claim 6, Eads et al. teaches a probe that has two signal components that are [in] proximity to one another (p. ii 2nd column 2nd paragraph).

With regard to Claim 7, Eads et al. teaches quencher-fluorescent dye pair (p. ii 2nd column 2nd paragraph).

With regard to Claim 17, Eads et al. teaches that several sequences are simultaneously amplified (p. ii 2nd column 2nd and 3rd paragraphs).

With regard to Claim 29, Eads et al teaches a method of using for diagnosing mismatching in genes associated with cancer disorders (abstract).

However, Eads et al. does not teach that the primer is joined with a probe via a linker.

Solinas et al. teaches using Scorpion primers in PCR assays (abstract). With regard to Claim 1, Solinas et al. teaches a primer whose 5' end is joined with a probe via a liner (e.g. Scorpion primer)(Figure 1).

With regard to Claim 8, Solinas et al. teaches the probe forms a hairpin shape (p. 1 2nd column 1st paragraph).

With regard to Claim 9, Solinas et al. teaches the probe bears two signal components separated in the inactive form and activated after hybridization (Figure 1A).

With regard to Claim 10, Solinas et al. teaches detecting the signal using FRET (p. 3 1st column 1st paragraph).

With regard to Claim 11, Solinas et al. teaches a duplex Scorpion format wherein there is a signal on the probe and a signal on another oligonucleotide (Figure 1B).

With regard to Claim 12, Solinas et al. teaches detecting the signal using FRET (p. 3 1st column 1st paragraph).

With regard to Claim 13, Solinas et al. teaches a duplex Scorpion format wherein there is a signal on the probe and a signal on

another oligonucleotide thereby separating the signals in the inactive form (Figure 1B).

With regard to Claim 14, Solinas et al. teaches a method wherein the probe comprises a signal and the other oligonucleotide bears a signal and under hybridization there is a signal (Figure 1b).

With regard to Claim 15, Solinas et al. teaches detecting the signal using FRET (p. 3 1st column 1st paragraph).

With regard to Claim 16 Solinas et al. teaches another binder binds in immediate proximity to the probe (Figure 1B).

With regard to Claim 18, Solinas et al. teaches that two scorpion primers can be used (Table 2).

With regard to Claim 19, Solinas et al. teaches that each Scorpion primer has a different signal (Table 2).

With regard to Claims 20, 21, and 28, Solinas et al. teaches Scorpion primers can be used to detect differences in nucleic acid structure (abstract). It would be obvious to design Scorpion primers to detect methylation and nonmethylated areas of the nucleic acid to use the primers in the methylation assay of Eads et al. Eads et al. teaches designing probes which hybridize to methylated and nonmethylated nucleic acid structures (Figure 1 of Eads et al.).

With regard to Claims 22-24, Solinas et al. teaches a probe with a quencher and a dye molecule which are in the inactive form when in spatial proximity (e.g. hairpin form) and are activated by hybridization of the probe to a primer extension product (Figure 1A).

With regard to Claims 25-27, Solinas et al. teaches an assay wherein the probe comprises a dye molecule and another oligonucleotides comprises a quencher and when the two are close they are inactive but after hybridization they are active (e.g. a duplex)(Figure 1B).

With regard to Claim 30, Solinas et al. teaches a method of using Scorpion primers to detect differences in tissues (abstract).

Therefore it would have been prima facie obvious to one of ordinary skill in the art to modify the methylation method of Eads et

> al. to include Scorpion primers linked to probes as taught by Solinas et al. The ordinary artisan would be motivated to modify the methylation method of Eads et al. to include Scorpion primers linked to probes as taught by Solinas et al. because Solinas et al. teaches the use of Scorpion primers in PCR assays allows an intermolecular probe-target interaction which results in a very fast and reliable detection system (p. 1 2nd column 2nd paragraph). Therefore the ordinary artisan would be motivated to use Scorpion primers to detect methylation and nonmethylation sites fast and reliably.

Insofar as the subject rejection relates to claims 29-30, Applicants respectfully submit that the rejection is moot in view of Applicants' cancellation herein of claims 29-30. Insofar as the subject rejection relates to claims 1-2 and 4-28, Applicants respectfully traverse the subject rejection.

Claim 1, from which claims 2 and 4-30 depend, has been amended herein and now recites "[a] method for the detection of cytosine methylations in DNA comprising the steps of:

a) treating the DNA to be investigated with a chemical or with an enzyme so that 5-methylcytosine remains unchanged, while unmethylated cytosine is converted to uracil or to another base which differs from cytosine in its base-pairing behavior,

b) amplifying the treated DNA of step (a) using a polymerase and at least one Scorpion primer, whose 5'-end is joined with a probe via a linker, whereby a primer extension product is produced,

c) separating the primer extension product from the matrix strand,

d) hybridizing the probe intramolecularly to the primer extension product, whereby the hybridization occurs as a function of the methylation state of the DNA,

e) detecting whether a hybridization of the probe has occurred."

As best understood by Applicants, the Patent Office is apparently taking the position (i) that Eads et al. teaches the general method of claim 1, except for the use of a Scorpion primer; (ii) that Solinas et al. teaches the use of a Scorpion primer in PCR assays; and (iii) that it would have been obvious to modify the method of Eads et al. by replacing the primer of Eads et al. with the Scorpion primer of Solinas et al.

For at least the reasons below, Applicants respectfully submit that there would have been no reason for one of ordinary skill in the art to make the modification proposed by the Patent Office. Therefore, Applicants respectfully disagree with the Patent Office's position that the claims are obvious.

First, the Scorpion primers of Solinas et al. are used only in connection with **genomic DNA** and only for the analysis of **SNPs or mutations**. By contrast, Eads et al. is directed at a method of **methylation analysis** that is used only in connection with **bisulfite-treated DNA**. The genomic DNA of Solinas et al. differs chemically and physically from the bisulfite-treated DNA of Eads et al., particularly in terms of its length, complexity, base composition and spatial structural. More specifically, during bisulfite treatment, DNA becomes strongly fragmented. The degree of the fragmentation depends on the condition of the reaction. The longer the reaction lasts, and the higher the temperature is, the greater is the decomposition rate of the DNA. On the other hand, long reaction times are necessary for a complete transformation. Therefore, bisulfite-treated DNA is presented as a complex mixture of fragments having different lengths and potentially incomplete transformed fragments. As the incomplete transformed DNA is a source of error for false-positive signals, it must be assured during analysis that only the transformed DNA will be detected.

Bisulfite-treated DNA also differs from genomic DNA in that bisulfite-treated DNA contains bases that are not present in genomic DNA. This is because, due to bisulfite treatment, all non-methylated cytosines are converted into uracil. Uracil is not present in genomic DNA. Although uracil shows the same base pair behavior as thymine, there are many enzymes that distinguish between uracil and thymine.

Moreover, bisulfite-treated DNA contains a totally different base composition than genomic DNA. Genomic DNA contains four bases. By contrast, bisulfite-treated DNA consists over large stretches of only three bases because all non-methylated cytosines are converted to uracil. These non-methylated cytosines represent at least all cytosines which are not in the sequence context CpG. Due to its 3-base alphabet, the bisulfite-treated DNA is less complex than the genomic DNA, and sequence repetitions can occur easier. Therefore, the design of specific primers or probes is much more difficult.

Furthermore, a signature feature of genomic DNA is its double helix structure. By contrast, bisulfite-treated DNA is not double-stranded, but rather, is single-stranded. Due to bisulfite treatment, two DNA strands are produced which are no longer complementary to one another and which no longer hybridize with one another. Moreover, due to its single-stranded structure, bisulfite-treated DNA is capable of forming intramolecular double-stranded segments.

In short, due to the many chemical and physical differences between genomic DNA and bisulfite-treated DNA, one of ordinary skill in the art would not have been motivated to use a Scorpion primer, which had previously been used only in connection with genomic DNA, in connection with bisulfite-treated DNA.

Nevertheless, despite a lack of motivation to use Scorpion primers in connection with bisulfite-treated DNA for methylation analysis, Applicants have noted several surprising advantages when using Scorpion primers for methylation analysis. First, the Scorpion technology is particularly well-suited for the analysis of short DNA fragments (as described in the present specification). This is important for several applications in methylation analysis, e.g., the analysis of DNA obtained from tissue samples or from bodily fluids. In these analytes, which are particularly well-suited for cancer diagnosis, the DNA is fragmented, either due to the fixation method (e.g., paraffin-embedded tissue samples) or due to biological reasons (e.g., DNA in bodily fluids often stems from apotosis or ageing processes and is thus fragmented). In addition, the bisuflite treatment leads to an additional fragmentation of the already short fragments (e.g., Grunau et al. 2001: Bisulfite genomic sequencing: systematic investigation of critical experimental parameters. Nucleic Acids Res. 2001 Jul 1; 29(13):E65). This is in contrast to SNP/mutation analysis, where no bisulfite treatment is conducted and, therefore, no additional fragmentation occurs. Thus, a powerful (i.e., sensitive and specific) diagnostic method based on DNA methylation must have the capabilty to analyze SHORT fragments. The application of the real time PCR methods known in the art (Lightcycler/Taqman) for this purpose is restricted. Lightcyler needs larger fragments, as two probes have to hybridize at adjacent positions. Also, for Taqman, one would usually need fragments > 80 bp. By contrast, Scorpion is also able to analyze shorter fragments.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claim 3 stands rejected under 35 U.S.C. 103(a) "as being unpatentable over Eads et al. (Nucleic acids Research 2000 Vol. 28 p. e32) in view of Solinas et al. (Nucleic acids Research 2001

Vol. 29 p. e96) as applied to Claims 1-2 and 4-30 and in further view of Berlin et al. (US Patent application Publication 2006/0183128 August 17, 2006)." In support of the rejection, the Patent Office states the following:

> The combination of Eads et al. and Solinas et al. teaches a method for detection of cytosine methylations in DNA, however, Eads et al. and Solinas et al. do not teach the addition of cytidine deaminase.
>
> Berlin et al. teaches a method of DNA methylation. With regard to Claim 3, Berlin et al. teaches cytidine deaminase to use in methylation reaction (paragraph 166 p 17).
>
> Therefore it would have been prima facie obvious to one of ordinary skill in the art to modify the methylation method of Eads et al. and Solinas et al. to include the reaction of cytidine deaminase as taught by Berlin et al. The ordinary artisan would be motivated to modify the methylation method of Eads et al. and Solinas et al. to include a reaction step with cytidine deaminase because Berlin et al. teaches that cytidine deaminase will convert cytosine bases which are unmethylated at the 5' position to uracil to differentiate between methylated and unmethylated cytosine bases (paragraph 166 p. 17). The ordinary artisan would be motivated to treat the DNA with cytidine deaminase such that there is a detectable difference between methylated and unmethylated cytosine bases.

Applicants respectfully traverse the subject rejection. Claim 3 depends from claim 1. Claim 1 is patentable over Eads et al. in view of Solinas et al. for at least the reasons given above. Berlin et al. fails to cure all of the deficiencies of Eads et al. and Solinas et al. with respect to claim 1. Therefore, based at least on its dependency from claim 1, claim 3 is patentable over the applied combination of Eads et al., Solinas et al. and Berlin et al.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claims 1-2 and 4-30 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Distler et al. (US Patent Application 2004/0265814) in view of Solinas et al. (Nucleic acids Research 2001 Vol. 29 p. e96)." In support of the rejection, the Patent Office states the following:

> Distler et al. teaches a method for detecting of cytosine methylation (abstract).
>
> With regard to Claim 1, Distler et al. teaches reacting the DNA with a chemical (e.g., sodium bisulfite) to change unmethylated cytosine to uracil, amplifying with a PCR amplification with a polymerase, at least one primer, and a probe (p. 3 paragraphs 28-32). However, Distler et al. does not teach that the primer is joined with a probe via a linker. With regard to Claim 1 step c-e, Distler et al. teaches separating the primer strand and detection whether or not hybridization of the probe has occurred (p. 3 paragraph 32).
>
> With regard to Claim 2, Distler et al. teaches reacting the DNA with sodium bisulfite (p. 3 paragraph 29).
>
> With regard to Claims 4-5, Distler et al. teaches a method of PCR (p. 3 paragraph 30).
>
> With regard to Claim 17, Distler et al. teaches that several sequences are simultaneously amplified (p. 2 paragraph 18).
>
> With regard to Claim 29, Distler et al teaches a method of using for diagnosing mismatching in genes associated with cancer disorders (p. 4 paragraph 45).
>
> However, Distler et al. does not teach that the primer is joined with a probe via a linker.
>
> Solinas et al. teaches using Scorpion primers in PCR assays (abstract). With regard to Claim 1, Solinas et al. teaches a primer whose 5' end is joined with a probe via a liner (e.g. Scorpion primer)(Figure 1).
>
> With regard to Claim 8, Solinas et al. teaches the probe forms a hairpin shape (p. 1 2nd column 1st paragraph).

With regard to Claim 9, Solinas et al. teaches the probe bears two signal components separated in the inactive form and activated after hybridization (Figure 1A).

With regard to Claims 6-7 and 10, Solinas et al. teaches detecting the signal using FRET (p. 3 1st column 1st paragraph).

With regard to Claim 11, Solinas et al. teaches a duplex Scorpion format wherein there is a signal on the probe and a signal on another oligonucleotide (Figure 1B).

With regard to Claim 12, Solinas et al. teaches detecting the signal using FRET (p. 3 1st column 1st paragraph).

With regard to Claim 13, Solinas et al. teaches a duplex Scorpion format wherein there is a signal on the probe and a signal on another oligonucleotide thereby separating the signals in the inactive form (Figure 1B).

With regard to Claim 14, Solinas et al. teaches a method wherein the probe comprises a signal and the other oligonucleotide bears a signal and under hybridization there is a signal (Figure 1b).

With regard to Claim 15, Solinas et al. teaches detecting the signal using FRET (p. 3 1st column 1st paragraph).

With regard to Claim 16 Solinas et al. teaches another binder binds in immediate proximity to the probe (Figure 1B).

With regard to Claim 18, Solinas et al. teaches that two scorpion primers can be used (Table 2).

With regard to Claim 19, Solinas et al. teaches that each Scorpion primer has a different signal (Table 2).

With regard to Claims 20, 21, and 28, Solinas et al. teaches Scorpion primers can be used to detect differences in nucleic acid structure (abstract). It would be obvious to design Scorpion primers to detect methylation and nonmethylated areas of the nucleic acid to use the primers in the methylation assay of Eads et al. Eads et al. teaches designing probes which hybridize to methylated and nonmethylated nucleic acid structures (Figure 1 of Eads et al.).

> With regard to Claims 22-24, Solinas et al. teaches a probe with a quencher and a dye molecule which are in the inactive form when in spatial proximity (e.g. hairpin form) and are activated by hybridization of the probe to a primer extension product (Figure 1A).
>
> With regard to Claims 25-27, Solinas et al. teaches an assay wherein the probe comprises a dye molecule and another oligonucleotides comprises a quencher and when the two are close they are inactive but after hybridization they are active (e.g. a duplex)(Figure 1B).
>
> With regard to Claim 30, Solinas et al. teaches a method of using Scorpion primers to detect differences in tissues (abstract).
>
> Therefore it would have been prima facie obvious to one of ordinary skill in the art to modify the methylation method of Distler et al. to include Scorpion primers linked to probes as taught by Solinas et al. The ordinary artisan would be motivated to modify the methylation method of Distler et al. to include Scorpion primers linked to probes as taught by Solinas et al. because Solinas et al. teaches the use of Scorpion primers in PCR assays allows an intermolecular probe-target interaction which results in a very fast and reliable detection system (p. 1 2nd column 2nd paragraph). Therefore the ordinary artisan would be motivated to use Scorpion primers to detect methylation and nonmethylation sites fast and reliably.

Insofar as the subject rejection relates to claims 29-30, Applicants respectfully submit that the rejection is moot in view of Applicants' cancellation herein of claims 29-30. Insofar as the subject rejection relates to claims 1-2 and 4-28, Applicants respectfully traverse the subject rejection.

Claims 1-2 and 4-28 are patentable over Distler et al. and Solinas et al. for at least the same types of reasons discussed above in connection with the rejection of claims 1-2 and 4-28 over Eads et al. and Solinas et al.

Furthermore, Applicants respectfully submit that Distler et al. is not even available as prior art against the present application. This is because the present application is a U.S. national entry of

PCT Application No. PCT/DE2004/001837, which was filed August 13, 2004. As a result, the present application has a filing date of August 13, 2004. By contrast, Distler et al. was not published until December 30, 2004, i.e., several months after the aforementioned August 13, 2004 filing date of the present application. Furthermore, Distler et al. is not entitled to a date as a 102(e) reference that is earlier than its December 30, 2004 publication date since Distler et al. is the U.S. national entry of an International Application filed on or after November 29, 2000, which International Application was **not** published in English.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claim 3 stands rejected under 35 U.S.C. 103(a) "as being unpatentable over Distler et al. (US Patent Application 2004/0265814) in view of Solinas et al. (Nucleic acids Research 2001 Vol. 29 p. e96) as applied to Claims 1-2 and 4-30 and in further view of Berlin et al. (US Patent Application Publication 2006/0183128 August 17, 2006)." In support of the rejection, the Patent Office states the following:

> The combination of Distler et al. and Solinas et al. teaches a method for detection of cytosine methylations in DNA, however, Distler et al. and Solinas et al. do not teach the addition of cytidine deaminase.
>
> Berlin et al. teaches a method of DNA methylation. With regard to Claim 3, Berlin et al. teaches cytidine deaminase to use in methylation reaction (paragraph 166 p 17).
>
> Therefore it would have been prima facie obvious to one of ordinary skill in the art to modify the methylation method of Distler et al. and Solinas et al. to include the reaction of cytidine deaminase as taught by Berlin et al. The ordinary artisan would be motivated to modify the methylation method of Distler et al. and Solinas et al. to include a reaction step with cytidine deaminase because Berlin et al. teaches that cytidine deaminase will convert cytosine bases which are unmethylated at the 5' position to uracil to differentiate between

> methylated and unmethylated cytosine bases (paragraph 166 p. 17). The ordinary artisan would be motivated to treat the DNA with cytidine deaminase such that there is a detectable difference between methylated and unmethylated cytosine bases.

Applicants respectfully traverse the subject rejection. Claim 3 depends from claim 1. Claim 1 is patentable over Distler et al. in view of Solinas et al. for at least the reasons given above. Berlin et al. fails to cure all of the deficiencies of Distler et al. and Solinas et al. with respect to claim 1. Therefore, based at least on its dependency from claim 1, claim 3 is patentable over the applied combination of Dislter et al., Solinas et al. and Berlin et al.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claims 1-30 stand provisionally rejected "on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claim 1-4, 15-16, 18 of copending Application No. 11716207 in view of Solinas et al. (Nucleic acids Research 2001 Vol. 29 p. e96)."

In response to the above, Applicants respectfully request that the subject provisional double patenting rejection be held in abeyance at least until the Patent Office has allowed one of the two patent applications at issue.

Claims 1-2 and 4-30 stand provisionally rejected "on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claim 1-2, 11, 14, 18-19 and 27 of copending Application No. 10482433 in view of Solinas et al. (Nucleic acids Research 2001 Vol. 29 p. e96)."

In response to the above, Applicants respectfully request that the subject provisional double patenting rejection be held in abeyance at least until the Patent Office has allowed one of the two patent applications at issue.

In conclusion, it is respectfully submitted that the present application is now in condition for allowance. Prompt and favorable action is earnestly solicited.

If there are any fees due in connection with the filing of this paper that are not accounted for, the Examiner is authorized to charge the fees to our Deposit Account No. 11-1755. If a fee is required for an extension of time under 37 C.F.R. 1.136 that is not accounted for already, such an extension of time is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

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By: _____

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on May 29, 2008

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Edward M. Kriegsman